LESSON 6:
TYPES OF CULTURES

Introduction

Cultures are generally initiated from sterile pieces of a whole plant. These pieces are termed 'explants' (already explained in previous lesson) and may consist of pieces of organs, such as leaves or roots, or may be specific cell types, such as pollen or endosperm. Many features of the explant are known to affect the efficiency of culture initiation. Generally, younger, more rapidly growing tissue (or tissue at an early stage of development) is most effective. Several different culture types most commonly used in plant transformation studies will now be examined in more detail. This will give you a general idea about the basic culture types used in tissue culture studies.

Callus

What are the Characteristic Features of Callus?

Explants, when cultured on the appropriate medium, usually with both an auxin and a cytokinin, can give rise to an unorganised, growing and dividing mass of cells called callus (Fig. 1). It is thought that any plant tissue can be used as an explant, if the correct conditions are found. In culture, this proliferation can be maintained more or less indefinitely, provided that the callus is subcultured on to fresh medium periodically. During callus formation there is some degree of dedifferentiation (i.e. the changes that occur during development and specialization are, to some extent, reversed), both in morphology (callus is usually composed of unspecialised parenchyma cells) and metabolism (renewed and enhanced RNA and protein syntheses). One major consequence of this dedifferentiation is that most plant cultures lose the ability to photosynthesise. This has important consequences for the culture of callus tissue, as the metabolic profile will probably not match that of the donor plant. This necessitates the addition of other components such as vitamins and, most importantly, a carbon source to the culture medium, in addition to the usual mineral nutrients. Callus culture is often performed in the dark (the lack of photosynthetic capability being no drawback) as light can encourage differentiation of the callus. During long-term culture, the culture may lose the requirement for auxin and/or cytokinin. This process, known as 'habituation', is common in callus cultures from some plant species (such as sugar beet). Callus cultures are extremely important in plant biotechnology. Manipulation of the auxin to cytokinin ratio in the medium can lead to the development of shoots, roots or somatic embryos from which whole plants can subsequently be produced. Callus cultures can also be used to initiate cell suspensions, which are used in a variety of ways in plant transformation studies (Fig. 1.).

Cell-Suspension Cultures

Callus cultures, broadly speaking, fall into one of two categories: compact or friable. In compact callus the cells are densely aggregated, whereas in friable callus the cells are only loosely associated with each other and the callus becomes soft and breaks apart easily. Friable callus provides the inoculum to form cell-suspension cultures. Explants from some plant species or particular cell types tend not to form friable callus, making cell-suspension initiation a difficult task. The friability of callus can sometimes be improved by manipulating the medium components or by repeated subculturing. The friability of the callus can also sometimes be improved by culturing it on 'semi-solid' medium (medium with a low concentration of gelling agent). When friable callus is placed into a liquid medium (usually the same composition as the solid medium used for the callus culture) and then agitated, single cells and/or small clumps of cells are released into the medium. Under the correct conditions, these released cells continue to grow and divide, eventually producing a cell-suspension culture. A relatively large inoculum should be used when initiating cell suspensions so that the released cell numbers build up quickly. The inoculum should not be too large though, as toxic products released from damaged or stressed cells can build up to lethal levels. Large cell clumps can be removed during subculture of the cell suspension.

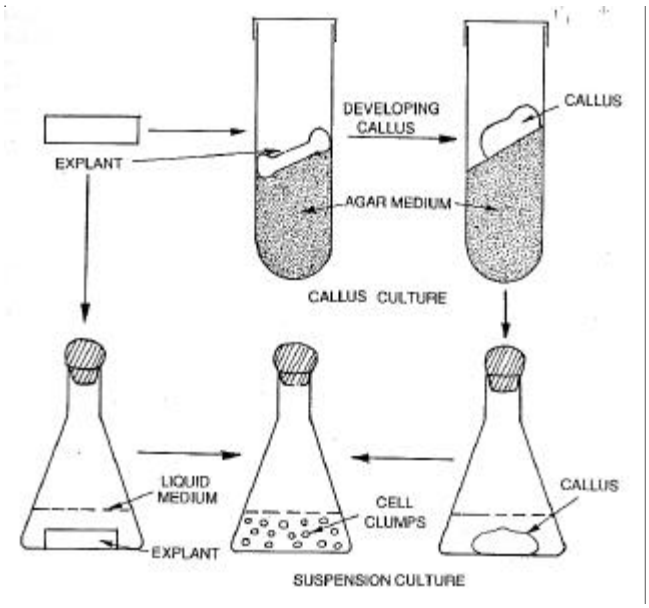

Fig. 1. Initiation of callus and suspension cultures.

Liquid cultures must be constantly agitated, generally by a gyratory shaker at 100-250 rpm (revolution per minute), to facilitate aeration and dissociation of cell clumps into smaller pieces. Suspension cultures grow much faster than callus cultures, need to be subcultured about every week, allow a more accurate determination of the nutritional requirements of cells and are the only system amenable to scaling up for a large scale production of cells and even somatic embryos (SEs).

The suspension cultures are broadly grouped as: (1) batch cultures, (2) continuous cultures, and (3) immobilized cell cultures.

Batch Cultures: In a batch culture the same medium and all the cells produced are retained in the culture vessel, e.g., culture flasks (100-250 ml), fermenters (variable size) etc. The cell number or biomass of a batch culture exhibits a typical sigmoidal curve (Fig.2), having a lag phase during which the cell number, or biomass remains unchanged, followed by a logarithmic (log) phase when there is a rapid increase in cell number and finally ending in a stationary phase during which cell number does not change. The lag phase duration depends mainly on inoculum size and growth phase of the culture from which inoculum is taken. The log phase lasts about 3-4 cell generations (time taken for doubling of cell number), and the duration of a cell generation may vary from 22-48 hr mainly depending on the plant species. The stationary phase is forced on the culture by a depletion of the nutrients and possibly due to an accumulation of cellular wastes. If the culture is kept in stationary phase for a prolonged period the cells may die.

Batch cultures are maintained by subculturing. They are used for initiation of cell suspensions which may be used for cloning, cell selection or as seed cultures for scaling up or for continuous cultures. They are, however, unsuitable for studies on cell growth and metabolism because there is a constant change in cell density and nutritional status of the medium. But batch cultures are much more convenient than continuous cultures and as a result, are routinely used.

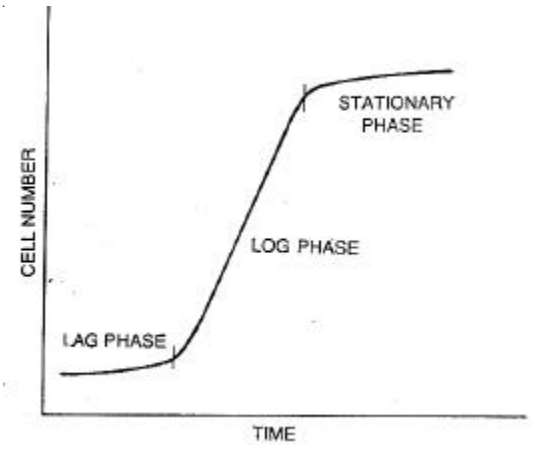

Fig. 2. A model curve for cell number in a batch culture.

Continuous Cultures: In a continuous culture, the cell population is maintained in a steady state by regularly replacing a portion of the used or spent medium by fresh medium. Such culture systems are of either (1) closed or (2) open type. In a closed continuous culture, cells are separated from the used medium taken out for replacement, and added back to the culture/so that cell biomass keeps on increasing. In contrast, both cells and the used medium are taken out from open continuous cultures and replaced by equal volume of fresh medium. The replacement volume is so adjusted that cultures remain at submaximal growth indefinitely.

The open cultures are of either turbidostat or chemostat types. In a turbidostat, cells are allowed to grow upto a preselected turbidity (usually measured as OD) when a predetermined volume of the culture is replaced by fresh normal culture medium. But in a chemostat, a chosen nutrient is kept in a concentration so that it is depleted very rapidly to become growth limiting, while other nutrients are still in concentrations higher than required. In such a situation, any addition of the growth-limiting nutrient is reflected in cell growth. Chemostats are ideal for the determination of effects of individual nutrients on cell growth and metabolism.

Immobilized Cell Cultures. Plant cells and cell groups may be encapsulated in a suitable material, e.g., agarose and calcium alginate gels, or entrapped in membranes or stainless steel screens. The gel beads containing cells may be packed in a suitable column or, alternatively, cells may be packed in a column of a membrane or wire cloth. Liquid medium is continuously run through the column to provide nutrients and aeration to cells. Immobilization of cells changes their cellular physiology in comparison to suspension culture cells; this offers several advantages for their use in biochemical production, but they are usually not used for other studies.

Subculture

What do you Understand by Subculturing?

After a period of time, it becomes necessary, chiefly due to nutrient depletion and medium drying, to transfer organs and tissues to fresh media. This is particularly true of tissue and cell cultures where a portion of tissue is used to inoculate new culture tubes or flasks; this is known as subculturing. In general, callus cultures are subcultured every 4-6 weeks, while suspension cultures need to be subcultured every 3-14 days. Plant cell and tissue cultures may be maintained indefinitely by serial subculturing.

In case of suspension cultures, subculturing should be done about or somewhat prior to the time of their maximum growth. The inoculum volume should be 20-25% of the fresh medium volume; in any case, the initial cell density of the fresh culture (just after inoculation) should be 5 x 104 cells /ml or higher otherwise the cells may fail to divide.

Selection of A Suitable Medium. A suitable medium may be devised for a new system in several ways. A simple approach is to first test-several concentrations, e.g., 0, 0.5, 2.5, 5 and 10m mol 1^{-1} of an auxin and a cytokinin to identify a suitable combination of the two. Now, first different auxins and then different cytokinins available to the worker may be tested to identify the best of each. Using these GRs, the different standard recipes may be evaluated. One may then check the 1/2, full and even higher salt concentration of the selected medium as well as different (2-6%) sucrose concentrations. A further refinement requires testing 1/2, full and x 2 concentrations of individual components of the selected recipe. Alternatively, the worker may evaluate the various combinations of low, medium and high concentrations of four solutions, i.e., minerals, organics, auxin and cytokinin, to arrive at a suitable recipe; De Fossard et al. (1974) have provided a broad spectrum experiment for this purpose (see, Bhojwani and Razdan, 1983).

Estimation of Growth

In order to make out if some growth has occurred in the culture, we follow certain methods like:

Cell number is the most informative measure of cell growth. This measurement is applicable to only suspension cultures, and even there cell aggregates must be treated, e.g., with pectinase, to dissociate them into single cells before counting the cell number in a haemocytometer. Therefore, cell number is estimated only where information obtained justifies the efforts. In contrast, packed cell volume of suspension cultures is easily determined by pipetting a known volume into a 15 ml graduated centrifuge tube, spinning at 200 x g for 5 min and reading the volume of cell pellet which is expressed as ml cells/l of culture.

Culture fresh and dry weights are the most commonly used measures of growth of both suspension and callus cultures. In case of callus cultures, the cell mass is placed on a preweighed dry filter paper or nylon filter and weighed to determine fresh weight. Cells from suspension cultures are filtered onto a filter paper or nylon filter, washed with distilled water, excess water removed under vacuum and weighed alongwith the filter; the filter is preweighed in wet condition. For dry weight determination the cells and the filter are dried in an oven at 60°C for 12 hr and weighed; the filter is pre-weighed in dry condition. Cell fresh and dry weights may either be expressed as per ml (suspension culture) or per culture.

Nuclear Cytology

This is done to understand changes occurring at the nuclear (chromosome and gene) level. Callus and suspension culture show both numerical (polyploidy and aneuploidy) and structural (deletions, translocations etc.) chromosome changes. The frequency of these changes tends to increase with time so that some cultures may become predominantly or even completely polyploid or aneuploid. Explants contain endopolyploid cells which may give rise to a portion of polyploid cells in cultures. But most polyploid cells appear to originate through endoreduplication (additional rounds of DNA replication without intervening cell division) although selection for such cells can not be ruled out.

Aneuploid cells originate mainly due to anaphase irregularities like unequal chromatid separation, lagging chromatids or chromosomes, anaphase bridges giving rise to breakage-fusion-bridge cycle, chromosome fragmentation etc. The cytogenetic status of cultured cells is influenced by several factors of the culture system, e.g., GR concentrations and combination, culture age, liquid or agar medium, subculture interval, sucrose concentration etc. Suspension cultures of many diploid species show a selection for diploid cells so that they remain predominantly diploid for long period, e.g., Vicia hajastana and Haplopappus gracilis cultures for over 300 days.

The above text gives you a basic idea about the concept of callus and suspension cultures. The following information and the coming Lessons will provide detailed studies on various kinds of cultures like:

Root Cultures

Root cultures can be established in vitro from explants of the root tip of either primary or lateral roots and can be cultured on fairly simple media. The growth of roots in vitro is potentially unlimited, as roots are indeterminate organs. Although the establishment of root cultures was one of the first achievements of modern plant tissue culture, they are not widely used in plant transformation studies.

Shoot Tip and Meristem Culture

The tips of shoots (which contain the shoot apical meristem) can be cultured in vitro, producing clumps of shoots from either axillary or adventitious buds.

This method can be used for clonal propagation (discussed later).

Shoot meristem cultures are potential alternatives to the more commonly used methods for cereal regeneration as they are less genotype-dependent and more efficient (seedlings can be used as donor material).

Embryo Culture

Embryos can be used as explants to generate callus cultures or somatic embryos.

Both immature and mature embryos can be used as explants. Immature, embryo-derived embryogenic callus is the most popular method of monocot plant regeneration (discussed later).

Conclusion

Establishment of single cell cultures provides an excellent opportunity to investigate the properties and potentialities of plant cells. Such systems contribute to our understanding of the interrelationships and complementary influences of cells in multicellular organisms. The single cell systems have a great potential for crop improvement. Free cells in cultures permit quick administration and withdrawal of diverse chemicals/substances, thereby making them easy targets for mutant selection. Moreover, the individual cells within a population of cultured cells invariably show cytogenetical and metabolic variations depending on the stage of the growth cycle and culture conditions. Therefore, it is essential to understand the various types of cultures in vitro and also their growth patterns.

Questions

1. What is an explant? How will you induce callus from it?
2. How will you prepare cell suspension ? Discuss the benefits of using aqueous media over the solid one.
3. Give a brief account of the growth pattern observed in batch cultures.
4. What methods would you employ to estimate cell growth in cultures? Discuss.

Note